a suppression effector that suppresses the expression of a mutant allele; and

a replacement nucleic acid which differs from the mutant allele in at least one degenerate / wobble position of at least one codon and wherein the replacement nucleic acid encodes a wild-type or non-disease causing protein.--

REMARKS

Claims 1-11 were pending in the Application. Upon entry of the present Amendment, claims 1-11 are cancelled and claims 12-44 are pending and presented for consideration. As quoted below, Applicants respectfully submit that no new matter is introduced by the present Amendment. The Specification has been amended to insert SEQ ID NOS and to correct typographical and grammatical errors. A substitute specification is provided. In order to expedite prosecution of this case and prompt allowance of the claims, Applicants address each of the Examiner's rejections from the second Office Action to the extent they are relevant to the new claims.

Support for Applicants' Claim Amendments

Applicants provide below a table which provides exemplary locations within the specification as originally filed at which support is provided for the claim amendments. Notwithstanding the table, Applicants respectfully contend that additional support for the claims can be found throughout the specification.

Claim	Support	Claim	Support
12	P. 8, line 11 to p. 9, line 3; p. 12, line 4 to p.13, line 15	29	P. 2, lines 26-31 P. 11, line 11 to P. 12, line 28 P. 19, lines 27 to P. 20, line 1 P. 21, lines 7-26
13	P. 2, lines 26-31 P. 12, lines 4-28 P. 19, line 27 to P. 20, line 1 P. 21, lines 7-26	30	P. 10, lines 16-20 P. 15, lines 26-30

14	P. 10, lines 16-20 P. 15, lines 26-30	31	P. 10, lines 16-20 P. 15, lines 25-30
15	P. 10, lines 16-20 P. 15, lines 26-30	32	P. 10, lines 16-20 P. 15, lines 26-30
16	P. 15, lines 25-30	33	P. 15, lines 25-30
17	P. 15, lines 25-30	34	P. 17, lines 24-34
18	P. 17, lines 24-34	35	P. 15, lines 26-30
19	P. 15, lines 26-30	36	P. 8, lines 19-22
20	P. 8, lines 19-22	37	P. 11, lines 19-23; p. 14, lines 6-20
21	P. 11, lines 19-23; p. 14, line 6-20	38	P. 10, lines 22-33
22	P. 10, lines 22-33	39	P. 15, line 25 to P. 17, line 5
23	P. 15, line 25 to p. 17, line 5	40	P. 16, lines 9-28
24	P. 16, lines 9-28	41	P. 16, lines 12-17 P. 17, lines 7-9 P. 37, line 24 to P. 42, line 11
25	P. 16, lines 12-17 P. 17, lines 7-9 P. 37, line 24 to P. 42, line 11	42	P. 14, lines 6-20
26	P. 14, lines 6-20	43	P. 11 lines 19-23 P. 14, lines 34-35
27	P. 11, lines 19-23 P. 14, lines 34-35	44	P. 15, lines 5-12
28	P. 8, line 11 to P. 9, line 3 P. 12, line 4 to P. 13, line 15		

Sequence Listing

Applicants enclose herewith an amended sequence listing, a computer readable form copy of the sequence listing, and a statement that the paper and computer readable

copies of the amended sequence listing are the same and that they include no new matter. No new matter is introduced by the amended sequence listing. Support for the amendments is provided below.

Sequence Amendment	**Support in the originally filed specification (PCT) PCT/GB97/00929**
SEQ ID NO:4: N=G at 135 and 137	Page 21, line 25, positions 35 and 37
SEQ ID NO:5: N=G at 135	Page 21, line 26, position 32
SEQ ID NO:12: delete N at 139	Page 23, line 34, after position 23
Introduce new SEQ ID NO:11	Page 23, line 2
Introduce new SEQ ID NO:12	Page 23, line 12
Introduce new SEQ ID NO:19	Page 44
Introduce new SEQ ID NO:20	Page 44
Introduce new SEQ ID NO:21	Page 44
Introduce new SEQ ID NO:22	Page 44
Introduce new SEQ ID NO:23	Page 44
Introduce new SEQ ID NO:24	Page 44
Introduce new SEQ ID NO:25	Page 44
Introduce new SEQ ID NO:26	Page 44
Introduce new SEQ ID NO:27	Page 44
Introduce new SEQ ID NO:28	Page 45

Applicants also enclose herewith a paper copy of the sequence listing, which was filed with the priority document WO 97/37014.

Rejections Under 35 U.S.C. §112, First Paragraph

The Examiner rejected claims 1-3 and 11 under 35 U.S.C. §112, first paragraph. The Examiner cited four references to support his contention that the ribozyme art is unpredictable. Applicants traverse the rejection to the extent it is applied to the new claims.

An analysis of enablement requires a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention. The test of enablement is whether one skilled in the art could make or use the claimed invention from the disclosures in the patent application coupled with information known in the art

without undue experimentation. *United States v. Telectronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); *In re Stephens*, 529 F.2d 1343 (CCPA 1976). Determining enablement is a question of law based on underlying factual findings. *In re Vaeck*, 947 F.2d 488, 495 (Fed. Cir. 1991); *Atlas Powder Co. v. E.I. duPont de Nemours & Co.*, 750 F.2d 1569 (Fed. Cir. 1984); MPEP 2164.01. Applicant may cite references to show what one skilled in the art knew at the time of filing the application. MPEP 2164.05.

Applicants submit that the specification fully enables new claims 12-44. Applicants provide detailed examples in their specification which provide the steps for practicing the invention. For example, the instant specification provides adequate guidance pertaining to (1) how to choose an appropriate target site for a ribozyme by analyzing RNA folding, (2) the design and construction of several ribozymes that reliably and reproducibly hybridize to and cleave RNA encoded by several mutant alleles - mammalian rhodopsin, peripherin and collagen genes - *in vitro*, and (3) the inability of the ribozyme to cleave replacement nucleic acids that are modified at degenerate / wobble base positions (e.g., third positions of a codon) and are thereby protected from suppression. For example, pages 19-20 of the Applicants' specification teach how a ribozyme target site can be chosen based on the presence of a ribozyme target sequence and the robustness of the two dimensional loop structure of the RNA in which the target sequence lies, as is determined by art known methods such as RNAPlotFold analysis. Pages 18-24 teach standard methods for cloning cDNA templates and ribozymes into a suitable expression vectors. Pages 25-34 teach detailed descriptions of the cleavage of exemplary mutations targeted by the exemplary ribozymes. The figures and results of Applicants' actual experiments on pages 37-49 teach that the exemplary ribozymes reproducibly cleave RNA comprising a ribozyme target site but do not cleave modified normal or non-disease-causing RNA, because the RNA does not contain the ribozyme target site. Applicants thus provide a method for designing suppression effectors, such as ribozymes, that destroy a target RNA and replace it with a replacement nucleic acid that has been altered in one or more degenerate / wobble bases. By exploiting the redundancy of the genetic code or the "wobble hypothesis", recognition or cleavage by the

suppression effector is blocked but a normal protein is encoded by the replacement nucleic acid.

Contrary to the Examiner's contention, there is no reason to believe that Applicants' method for designing the suppression of a mutant allele of a gene and providing a replacement allele, would not work *in vivo* for suppressing a gene, given the state of the art of gene therapy, antisense and ribozymes. Applicants respectfully direct the Examiner to *In re Wright*, 999 F.2d 1557, 1564 (Fed. Cir. 1993), which held that the standard of enablement is a "reasonable expectation of success". That is, the enablement requirement under §112 is met when there is a reasonable belief that Applicants' success with one embodiment of the invention could be extrapolated to other embodiments by a skilled artisan at the time of the effective filing date of an application. Applicants' invention can be used to alleviate autosomal dominant disease symptoms, even if 100% of the mutated gene product is not suppressed (Applicants' specification, page 16). Thus, Applicants believe that there would have been a reasonable expectation of success that their gene therapy strategy would work *in vivo* to alleviate at least one symptom of disease by at least lowering mutant RNA levels.

Applicants disagree with the Examiner's contention that the ribozyme art was unpredictable at the time of filing the instant application. Several methods in the article demonstrated that ribozymes could be designed (using 2^{o} structure modeling and testing in vitro) which then cleaved RNA in vivo. For example, Lieber and Strauss (1995) *Mol. & Cell. Biol.* 15:540-51 (attached as Exhibit A), describe a strategy for expression of ribozymes for selection of accessible cleavage sites in target RNAs and for the isolation of corresponding ribozymes for any mRNA of interest. In addition U.S. Patent No. 5,582,972 (attached as Exhibit B) provides methods for preparing antisense oligonucleotides which take advantage of RNA secondary and tertiary structure and with specificity that is not less than 1/1000 of its affinity for a length matched oligonucleotide complement. The effectiveness of these and other well known methods for ribozyme design were demonstrated for a number of *in vivo* applications. For example, Lieber and Kay (1996) J.Vir. 70:3153-58 (attached as Exhibit C) describe the use of a human growth hormone (hGH) ribozyme that was infused into transgenic mice and demonstrated up to

96% reduction in hepatic hGH mRNA. These investigations demonstrated that ribozymes can function in an intact organ after somatic gene transfer. U.S. Patent No. 5,246,921 (attached as Exhibit D) claims methods for treating a patient having a leukemia characterized by the presence of a hybrid oncogene with a ribozyme, wherein the cells were transfected *in vitro* and re-introduced back into the patient. U.S. Patent No. 5,834,440 (attached as Exhibit E) claims methods for inhibiting abnormal smooth muscle cell proliferation in vascular tissue by introducing a ribozyme into a cell and administering the ribozyme to a subject using exoluminal, transluminal, stent, biodegradable polymer, sphere or pleuronic gel.

Applicants disagree with the Examiner's contention that the invention would require undue experimentation to develop methods for *in vivo* suppression of any endogenous gene to produce the desired therapeutic effect. The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Angstadt*, 537 F.2d 498, 190 USPQ 214 (CCPA 1976); MPEP 2164.01; *M.I.T. v. A.B. Fortia*, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985). For example, the expression of ribozymes and their cleavage of target RNA in human cells was well known in the art at the time of the invention. Once transfected into cells by methods well known to the skilled artisan, the effectiveness of a ribozyme construct can be easily measured or monitored by, for example, determining mRNA or protein levels of the cell using standard methods of molecular biology.

Moreover, methods of introducing cells transfected with ribozymes into an animal for treatment of disease were also described in the literature at the time of filing. For example, the transfection of autologous cells with a ribozyme which are then replaced into the same host is a technique which was well known in the art and one which also was easily monitored. A number of detailed protocols for autologous cell transfection and transplantation were available in the literature published prior to Applicants' filing date, including those provided in U.S. patents issued prior to the Applicants' filing date. For example, U.S. Patent 5,399,346, which issued on March 21, 1995 (attached as Exhibit F), broadly claims "[a] process for providing a human with a therapeutic protein comprising: introducing human cells into a human, said human cells having been treated *in vitro* to

insert therein a DNA segment encoding a therapeutic protein said human cells expressing *in vivo* in said human a therapeutically effective amount of said therapeutic protein." U.S. Patent No. 5,087,617, which issued February 11, 1992 (attached as Exhibit G), claims methods for treating bone marrow cells from an individual having cancer prior to infusion of the bone marrow cells back into the individual. This patent provides examples which the PTO deemed enabling for *in vivo* use in patients (see e.g., independent claims 2, 4 and 6), as well as guidance to the skilled artisan for providing antisense to a patient.

Thus, many methods for designing ribozymes and / or introducing them, or genes generally, into humans were in the public domain before the filing date of the instant patent application such that a skilled artisan would have a reasonable expectation of success that such strategies would work *in vivo* without undue experimentation.

Applicants respectfully submit that the specification is sufficient to enable one having an ordinary skill in the art, given the high level of skill in the art at the filing date of the instant application, to make and use the invention as now claimed without undue experimentation. *See Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524 (Fed. Cir. 1987).

Rejections Under 35 U.S.C. §112, Second Paragraph

Applicants have cancelled claims 1-11 and have submitted new claims 12-44 which they believe obviate the §112 rejection. Applicants respectfully request that the rejections under 35 U.S.C. §112, second paragraph, be withdrawn and that the Examiner consider the new set of claims.

Rejections Under 35 U.S.C. §102

The Examiner rejected claims 2-3 under 35 U.S.C. §102(b) as being anticipated by Hart et al. (1995) Human Mol. Genet. 4(9):1597-1602 (hereinafter "Hart"). Applicants traverse the rejection to the extent it is maintained over the new claims.

For anticipation under 35 U.S.C. §102, the reference must teach every aspect of the claimed invention either explicitly or impliedly.

Hart teaches a method and an expression vector used to detect transgenic expression. Hart describes a double mutant cystic fibrosis transmembrane conductance regulator protein gene (*CFTR*) cDNA construct that allows the analysis of transgenic expression by digestion of RT-PCR products with two restriction enzymes which yield complementary information. Two oligonucleotide-directed point mutations were introduced into *CFTR* cDNA to destroy a *SphI* site in one instance and create an *AgeI* site in another.

Applicants' new claims recite compositions and methods that are designed to exploit the redundancy of the genetic code for downregulating mutant gene expression and replacing it with normal gene expression. Applicants used suppression effectors, such as ribozymes, to inactivate RNA encoded by a mutant allele, and normal or non-disease causing replacement nucleic acids wherein one or more degenerate / wobble bases are altered to inhibit suppression by the suppression effector. For example, if a mutant RNA comprises an NUX ribozyme target site, ribozyme suppression of the replacement nucleic acid, e.g. a replacement transcript which encode a non-disease causing protein, can be inhibited by altering either the N, the U, or the X, depending upon which of the N, U or X is at a degenerate / wobble position in a codon. In addition, replacement nucleic acids can be altered at degenerate/wobble positions in the sequence complementary to the ribozyme antisense arms. It is likely that in addition to suppressing a mutant allele, the other endogenous allele (e.g., normal or non-disease causing allele) will also be suppressed.) In the case of a disease where an individual has a dominant negative mutation in at least one allele of a gene which causes the disease, the invention allows one to design a suppression effector that suppresses the RNA having the mutation but which does not suppress a non-disease causing RNA encoded by replacement nucleic acid(s) that does not have the mutation.

Because the replacement nucleic acid is not suppressed by the suppression effector, both the suppression effector and the replacement nucleic acid can be introduced

at the same time. Suppression can be achieved by antisense nucleic acids, triple helix-forming nucleic acids, PNAs, antibodies, or other DNA, RNA or protein-binding molecules which specifically recognize (i.e., bind and/or cleave or otherwise suppress) a mutant variant of a gene or its product. The invention allows for suppression of a mutant allele in a mutation-independent manner and replacement with a normal or non-disease causing gene such that this replacement allele is not suppressed, using the degeneracy of the genetic code to alter the replacement gene. Many dominant disorders can be caused by a diverse range of different mutations in a given gene. This diversity is a significant hurdle for therapeutic development. The invention overcomes this hurdle by enabling the design and generation of a therapeutic agent for individuals irrespective of the mutation(s) that they harbor in a given gene.

Hart does not teach the claimed invention either explicitly or inherently. Hart does not teach suppression effectors. Hart does not describe downregulating endogenous gene expression. Hart therefore does not teach the problem that Applicants are trying to solve - e.g., the replacement of mutant gene expression. The Hart reference also does not teach replacement nucleic acids, the expression of which are not suppressed by suppression effectors or are only partially suppressed. Hart only describes a pair of mutations that are used to specifically detect their *CFTR* transgene expression among the endogenous mutant *CFTR* expression. Hart does not describe ribozymes which cleave RNA but uses restriction enzymes to cleave RT-PCR products. Hart does not, therefore, describe the construction of replacement nucleic acids by mutating the NUX cleavage site or the nucleotides to which one or both ribozyme arms bind. Hart describes only a diagnostic method which is useful to monitor the expression of an exogenous gene, not Applicants' method for downregulating endogenous mutant gene expression and replacing it with exogenous normal gene expression. In addition, Hart et al. does not teach about overcoming mutational diversity inherent in many disorders and the suppression and replacement of mutant genes utilizing the degeneracy of the genetic code. Hart was not developing therapies but was modifying wobble bases to act as a marker for gene transfections. In particular, Hart does not teach therapies for dominant negative genetic diseases. Hart does not teach suppression of a gene allele and therefore

does not teach replacement of the suppressed allele. In light of the foregoing arguments, Applicants respectfully request that the rejection under 35 U.S.C. §102 be reconsidered and withdrawn.

CONCLUSION

Applicants respectfully urge that all claims are in condition for allowance. Applicants request that the Examiner reconsider the new claims and prompt and favorable action on the instant application.

Request for a Telephonic Interview

Applicants hereby request a telephonic interview with the Examiner in order to expedite the favorable prosecution of the case. The Examiner is invited to phone the undersigned to arrange for a convenient time to discuss any outstanding issue, and to work with the Examiner toward placing the application in condition for allowance.

Applicants believe that no additional fees are necessitated by this Amendment. However, in the event that any additional fees are due, the Commissioner is hereby authorized to charge any such fees to Attorney's Deposit Account No. 20-0531.

Respectfully submitted,

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